DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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MEMORANDUM

DATE: September 12, 1997

FROM: Kathleen A. Clouse, Ph.D., Division of Cytokine Biology KAC
Malcolm Moos, M.D., Ph.D., Division of Cell and Gene Therapy MM

SUBJECT: Lukor pre-IND meeting

TO: Dr. Yash P. Sharma

ould you please bring the following additional materials to the pre-IND meeting scheduled to be held at CBER on September 18, 1997:

1. SDS-PAGE gels (reduced and non-reduced) on both human and baboon leukocyte extracts following precipitation with your stabilizing solution. These should be stained with both Coomassie blue and silver stain.

2 SDS-PAGE gel prepared as indicated above on HPLC purified baboon extract, if possible.

3. Additional product characterization and manufacturing data as discussed with Dr. Moos on September 11, 1997.

Please feel free to contact us if you have any questions

Original Paper

NEPHRON

Nephron 1996;72:599–603

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Chege J. Mukuria
Eiko Suzuki
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Failure of Human Immunoresponse to N-Glycolylneuraminic Acid Epitope Contained in Recombinant Human Erythropoietin

Key Words
Erythropoietin
Hanganutziu-Deicher antibody
N-glycolylneuraminic acid
Recombinant glycoprotein
Sialic acid
Hemodialysis

Abstract
Recombinant human erythropoietin (rHuEPO) was produced by Chinese hamster ovary cells and commercially distributed to hospitals by two pharmaceutical companies in Japan ('ESPO' by Kirin Brewery Co. Ltd., and Sankyo Co. Ltd., and 'EPOGIN' by Chugai Pharmaceutical Co. Ltd.) These products contained about 1% N-glycolylneuraminic acid (Neu5Gc) in total sialic acid content. Since humans do not synthesize Neu5Gc, successive injection of Neu5Gc-containing products was feared to lead to allergic-like symptoms. Therefore, serum levels of antibodies to Neu5Gc epitope in 90 patients who received repeated i.v. injections of ESPO or EPOGIN were determined by an enzyme immunoassay using Neu5Gcα2-3Galβ1-4Glc-Cer, GM3(Neu5Gc), as an antigen and compared with those in 100 healthy persons. Either no or low antibody levels were detected in both groups with no significant difference. In 40 patients who received s.c. injections of ESPO or EPOGIN, serum HD antibody levels were determined before and after weekly therapeutic injections carried out for one to several months, but no significant elevations were detected in all patients. The above results indicated that therapeutic administration of rHuEPO to patients with chronic renal failure is safe from allergic-like side effects associated with the production of Neu5Gc-specific antibodies, and it was concluded that Neu5Gc epitope of rHuEPO is minimally antigenic in humans.

Introduction

Erythropoietin (EPO) is a glycoprotein hormone which stimulates proliferation and differentiation of erythroid precursors in bone marrow. It is produced primarily by the kidney in response to renal hypoxia. For the recombinant glycoprotein to express its biological function, its glycosylation is necessary. Recombinant human EPO (rHuEPO) produced by bacteria lacks glycosylation of the molecule and that produced by yeasts contains a large amount of mannose-type N-linked carbohydrate chains. However, both rHuEPO lose their biological activities when they are administrated in vivo [1]. Therefore, Chinese hamster ovary (CHO) cells have been recently used to produce rHuEPO. The rHuEPO produced by CHO cells carries N-linked carbohydrate chains containing sialic acids at

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April 18, 1995

their nonreducing ends and they do exhibit biological activities. However, it was found that the recombinant products possess a small proportion of N-glycolyl neuraminic acid (Neu5Gc) type sialic acid [2] which is a foreign component in humans. The component should have antigenic capability and can, at least theoretically, give rise to an immunological response in humans.

Human chronic renal failure decreases serum EPO levels and causes chronic anemia. The biologically active rHuEPO was made available to patients with chronic renal failure in Japan (Commercial name, ESPO was produced by Kirin Brewery Co., Ltd. and marketed by Sankyo Pharmaceutical Co. Ltd. and EPOGIN was produced and sold by Chugai Pharmaceutical Co. Ltd. in 1990). So far, about 80,000 patients have received rHuEPO therapy in Japan, but no allergic incident has been reported.

In 6,300 patients who were intravenously administered with rHuEPO for the phase I–IV clinical trials, no elevation of rHuEPO-specific antibody levels was detected by a highly sensitive enzyme immunoassay and/or radioimmunoassay [3]. Recently, however, an anaphylactic reaction to rHuEPO took place in one Spanish patient during intravenous administration and where rHuEPO-specific IgE antibody was detected [4].

One of the antigenic determinants in rHuEPO may be Neu5Gc. Indeed, the immunization of chickens (who like humans do not synthesize Neu5Gc) with rHuEPO led to a production of low titer antibody against Neu5Gc epitope [5]. Human cancerous tissues and some chicken lymphomas express Neu5Gc in their sialoglycoconjugates, and this expression frequently stimulates the production of the so-called Hanganutziu-Deicher (HD) antibody which recognizes Neu5Gc moiety of the sialoglycoconjugates [6,7].

In the present study, we examined the HD antibody levels in sera from 90 patients receiving routine injections of rHuEPO. The result indicated that no case had a significantly high titer of HD antibody. In 40 patients, HD antibody titers before and after the start of the rHuEPO therapy did not change at all.

Materials and Methods

Materials

Two CHO cell-derived rHuEPO products, ESPO and EPOGIN were purchased from Sankyo Co. Ltd. and Chugai Pharmaceutical Co. Ltd., respectively. They were intravenously administered into 90 hemodialysis patients initially at a dose of 50–100 IU/kg body weight at each dialysis time and continued for at least 5 years. The dose was individually reduced or increased to a total dose of 1,500–3,000 IU within 2 weeks in order to keep the patient's hematocrit level at 30–35%.

Distribution of ages in the 90 patients was as follows: 12–19 years, 1 person (1%); 20–29 years, 5 persons (6%); 30–39 years, 16 persons (18%); 40–49 years, 29 persons (32%); 50–59 years, 26 persons (29); 60–73 years, 13 persons (14%). They suffered from renal failure, mainly of chronic glomerulonephritis.

In the phase II and III clinical trials, ESPO was administered subcutaneously to 14 patients. One with renal failure, 6 with intractable anemia such as aplastic anemia or myelodysphastic syndrome, and 7 with chronic rheumatoid arthritis. There were 6 other patients who had to provide their own blood for autotransfusion in future surgery. EPOGIN was also administered subcutaneously into 20 patients. Seventeen patients had chronic renal failure and 3 patients provided their own blood for autotransfusion. They received doses of 6,000–24,000 IU (per day or week) for 3 weeks to 8 months. Their serum HD antibody levels were determined before and after the therapy. Sera from 50 healthy persons were purchased from Mitsubishi Yuka BCL Co. Ltd. and those from another 50 healthy persons were obtained from donors who had no abnormal values in their clinical blood examinations. A HD antibody-positive serum was obtained from a patient with lung carcinoma and its antibody specificity was previously examined against various Neu5Gc-containing compounds [7].

GM3(Neu5Gc), Neu5Gcα2-3Galβ1-4Glc-Cer, and GM3(Neu5Ac), Neu5Acα2-3Galβ1-4Glc-Cer, where Neu5Ac is N-acetylneuraminic acid; Neu5Gc, N-glycolylneuraminic acid; Gal, *D*-galactose; Glc, *D*-glucose; Cer, Ceramide (N-acylsphingosine); were previously purified from horse erythrocytes and dog erythrocytes, respectively [6].

EIA

Serum antibody levels to GM3(Neu5Gc) were determined by EIA. For coating 96-microplate wells (Ts-Flat, Toyoshima Co.) with the antigen, 300 ng of GM3(Neu5Gc) in 50 μl methanol were added and completely evaporated in a 37 °C incubator. The microplate wells were washed 4 times with 0.01 *M* phosphate buffer (pH 7.0) containing 0.15 *M* NaCl (PBS) containing 0.05% Tween 20 and nonspecific sites were blocked by incubation with 200 μl of 1% egg albumin-PBS at 37 °C for 1 h. After similar washing, 50 μl of a test serum diluted 100-fold with 1% egg albumin-PBS was added and the reaction was done at 37 °C for 1 h. After similar washing, 50 μl of the 2nd antibody of peroxidase-conjugated antihuman immunoglobulin (Cappel Co.) at a dilution of 1:1,000 was added and reaction was carried out under the same conditions. After another washing, a substrate solution (200 μl) containing 200 μ*M* 2,2′ azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (Wako Pure Chemical Industries, Ltd.) and 0.015% H_2O_2 in 50 m*M* citrate buffer (pH 4.0) was added and incubated at 37 °C for 30 min. The absorbance of the produced color was read at 405 nm. The OD value of each nonantigen-coated well was subtracted from that of an antigen-coated well. A HD antibody-positive serum from a cancer patient was determined at a dilution of 1:400 in the same manner and at the same time as the test sera were assayed. This serum dilution gave an absorbance of about 1.0 at 405 nm.

A relative antibody titer of a test serum from a mean of 3 determination was calculated as follows:

$$\frac{OD_{405} \text{ of a test serum at 1:100 dilution}}{OD_{405} \text{ of a control serum at 1:400 dilution}} \times 100$$

Table 1. Changes of HD antibody titers in patients before and after rHuEPO therapy

ESPO			EPOGIN		
patient No.	before	after	patient No.	before	after
1	25.0[a]	1.4	21	58.8	37.6
2	23.2	35.4	22	51.4	45.8
3	14.2	18.2	23	47.1	38.8
4	9.7	3.2	24	44.9	44.0
5	6.7	20.0	25	40.1	53.4
6	5.7	19.1	26	23.1	15.3
7	5.7	10.1	27	3.2	14.1
8	5.1	6.0	28	0.7	none
9	3.9	7.8	29	none	7.0
10	3.8	6.3	30	none	2.6
11	3.7	6.0	31	none	none
12	3.6	2.0	32	none	none
13	2.3	none[b]	33	none	none
14	2.1	2.6	34	none	none
15	2.1	none	35	none	none
16	0.9	8.2	36	none	none
17	0.7	2.0	37	none	none
18	0.1	0.8	38	none	none
19	none	0.3	39	none	none
20	none	none	40	none	none

[a] Serum HD antibody titer was determined by EIA using GM3(Neu5Gc) as the antigen as described in 'Materials and Methods' and expressed as a relative value to a positive control titer (100).

[b] Antibody titer was negligible

EIA Inhibition Test

GM3(Neu5Gc) or GM3(Neu5Ac) dissolved in 1% egg albumin-PBS at a concentration of 1 μg/ml was mixed with the same volume of 50-fold diluted test serum in the same buffer and incubated at 37°C for 1 h. Then, 50 μl of the mixture was added to a GM3(Neu5Gc)-coated microplate well. The antibody titers before and after the absorption were triplicately determined as described above.

Results

Since GM3(Neu5Gc) was used as the antigen in this experiment, an EIA inhibition test was performed with ESPO and EPOGIN. A HD antibody, which was obtained from a patient with lung cancer, recognized the Neu5Gc epitope of ESPO and EPOGIN [5]. Therefore, it was confirmed that the EIA using GM3(Neu5Gc) as the antigen can detect antibodies to Neu5Gc epitope of ESPO and EPOGIN.

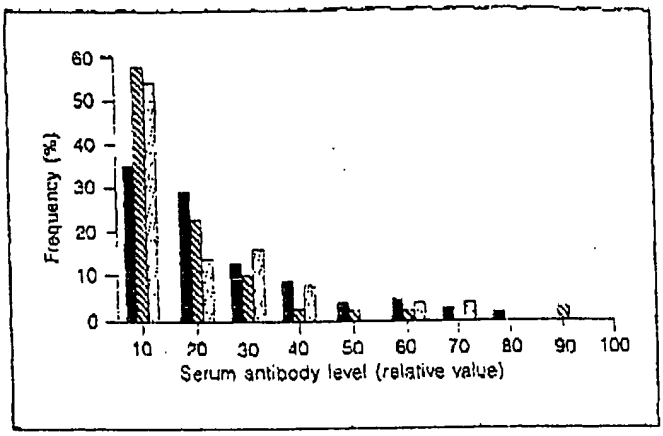

Fig. 1. HD antibody levels of sera from patients receiving rHuEPO injections and healthy persons. Serum HD antibody levels of 40 hemodialysis patients who had received intravenous injections of ESPO 2 or 3 times a week for a long term (▨) and those of 50 hemodialysis patients who had received the same doses of EPOGIN (▩) were compared with those of 100 healthy persons (■). The antibody level was determined by an EIA and expressed as a relative value to a positive control value (100) as described in 'Materials and Methods'. Frequency percentages at an interval of 10 of the relative value are illustrated. The relative value of 10 represents values between 0 and 10.

Serum HD antibody levels of 40 hemodialysis patients with renal disorder who were receiving ESPO for at least 6 years, those of 50 hemodialysis patients with the same disorder who were receiving EPOGIN for at least 5 years and those of 100 healthy persons were compared as shown in figure 1. The antibody titers for each group were not so high in comparison with those frequently found in cancerous patients [8] and those for both ESPO and EPOGIN groups were not significantly higher than those for the healthy group ($p > 0.05$).

Recently, subcutaneous administration of comparatively high doses of rHuEPO began in the 2nd and 3rd clinical trials. Blood samples were taken from 20 randomly selected patients who were to be administered ESPO and another 20 patients who were to receive EPOGIN in order to examine their HD antibody levels before the start of the trial and after the therapy had continued for 3 weeks to 8 months. However, the antibody levels did not change significantly during that period, as shown in table 1 ($p > 0.05$).

For confirmation of HD antibody specificity of patient and healthy person sera, an absorption test was carried out in 20 sera (6 from hemodialysis patients and 14 from healthy persons) which showed significant HD antibody

titers. In all sera, addition of GM3(Neu5Gc) inhibited the antibody activity by between 65 and 99%, but no inhibition was possible with GM3(Neu5Ac).

Discussion

Various recombinant human glycoproteins synthesized in CHO cells, such as γ-interferon [9], FSH [10], EPO [11, 12], plasminogen activator [13, 14], HIV gp120 [15], CD4 [16, 17] and interleukin-5 [18] have been reported to have N-linked oligosaccharides with mono-, di-, tri- and tetra-antennary chains. They have α2-3 sialyl lactosamine structures at their carbohydrate chain terminals. Some recombinant glycoproteins produced in CHO cells such as EPO [19] and interleukin-2 [20], also have O-linked oligosaccharide chains with α2-3 sialyl galactose and/or α2-6 sialyl N-acetylgalactosamine structure at their terminals. The total sialic acid content of recombinant plasminogen activator, EPO and FSH produced by CHO cells was reported to contain about 3% Neu5Gc [2], while the presently used rHuEPO preparations, ESPO and EPOGIN had 1% Neu5Gc. The Neu5Gc moiety is transferred from CMP-Neu5Gc into the terminal portions of the carbohydrate chains of these glycoproteins. CMP-Neu5Gc may be synthesized by CMP-Neu5Ac hydroxylase from CMP-Neu5Ac [21, 22] or free Neu5Gc may be synthesized from free Neu5Ac by Neu5Ac hydroxylase [23], and then converted to CMP-Neu5Gc by CMP-Neu5Gc synthase. Either or both hydroxylation systems should be present in CHO cells.

HD antibodies which recognize the Neu5Gc epitope of sialoglycoconjugates have been detected in sera from some cancerous patients and it was believed that this antibody response is a result of the immune system being exposed to a small amount of Neu5Gc epitope expressed on tumor cells [2, 3]. Thus, even 1% Neu5Gc as in rHuEPO may possibly stimulate antibody production and lead to an allergic-like serum sickness in patients. We therefore first immunized chickens with rHuEPO as a model system [5]. Two chickens responded weakly to the Neu5Gc epitope of rHuEPO but another did not respond significantly, while those immunized with fetuin, which contains 6% Neu5Gc, produced significantly high titers of HD antibodies. This immunization was carried out by one subcutaneous administration of the antigen with Freund's complete adjuvant. Human patients with chronic renal failure have to receive repeated injections of rHuEPO long term. Therefore, rHuEPO therapy still has the potential of causing allergic side effects in such patients. In the present study, we examined the HD antibody levels of sera from 90 hemodialysis patients who were receiving rHuEPO injections. Many of the sera (more than 50%) had negligible titers and others showed low titers, i.e. less than 100 in the relative value. HD antibody-positive sera from cancerous patients usually showed high titers of more than 100 [8]. The distribution of antibody titers in patients was not significantly different from that in 100 healthy persons. Subcutaneous injection of higher doses easily stimulates an antibody response. Therefore, 40 patients who were receiving subcutaneous injections of 6,000–24,000 IU (33–132 μg of rHuEPO) per day or weekly were examined, but no significant elevation of the HD antibody level was demonstrated during the continuous therapy of 3 weeks to 8 months.

The Neu5Gc situated in the carbohydrate portion of rHuEPO is highly antigenic but the peptide part may not be antigenic to humans. The Neu5Gc moiety itself is only a hapten which requires a carrier protein recognizable by human T cells in order to stimulate the production of Neu5Gc-specific antibody. So far, no antibody against rHuEPO has been detected by a highly sensitive enzyme immunoassay and/or radioimmunoassay in sera from 6,300 patients who received successive injections of rHuEPO [3]. Recently, one case was reported by Garcia et al. [4] that a patient produced rHuEPO-specific IgE antibody, but it is still unknown whether its antigenic epitope is either a carbohydrate or a peptide. Elucidation of the antibody binding site to rHuEPO will be important in further studies.

Acknowledgments

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References

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AMGEN

Professional Services Department

DATE: June 8, 2001

FACSIMILE COVER SHEET

PLEASE DELIVER IMMEDIATELY TO:

NAME: Yah Sharma

INSTITUTION: Medicine & Applied Sciences

TEL: 703-749-1794

FAX: 703-448-0536

FROM: Gabriel Mesa, MD

TEL: 805-447-5427

FAX: 805-376-8550

Pages: 1 (including cover)

Background information:

1. N-Glycolylneuraminic acid (NGNA) is one of the sialic acids (carbohydrates) present in EPO and ARANESP. The other one is acetylneuroaminic acid (NANA).

2. NGNA is a small molecule weighting 325 Daltons (Data not confirmed)

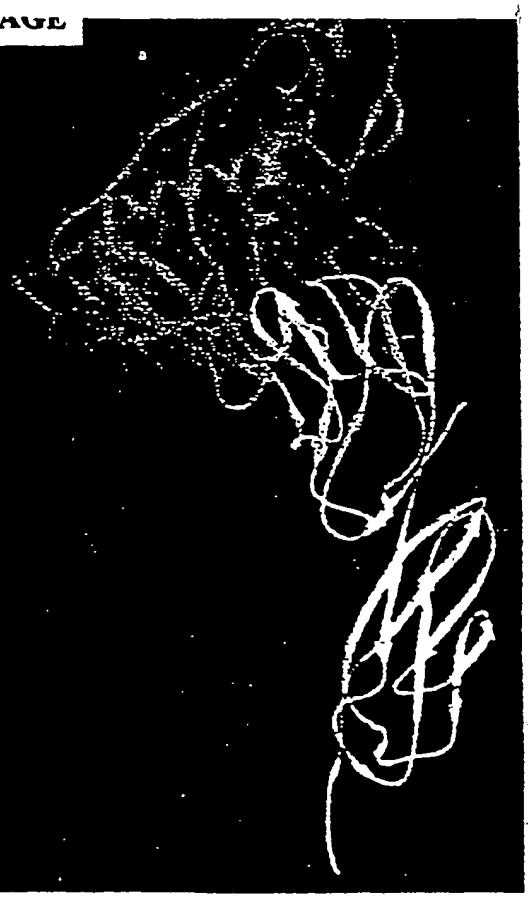
3. NGNA is not produced in humans, but it is present in many mammals eg. horses, Chinese hamsters, chimpanzees, baboons, etc. NANA is present in humans.

4. EPO contains 1% NGNA (see article by Nouguchi - enclosed).
   According to this, an average 70 kg pt receiving a dose of 100 u/kg TIW receives 1.8 µg of N-glycolylneuraminic acid/wk.
   (100 u Epo x 70 kg = 7,000u x 3 – 21,000 u Epo/wk = 0.18 mg Epo = 180 µg (119,000 u Epo = 1 mg Epo). 1% of 180 µg = 1.8 µg of NGNA/wk.)

5. The immunogenicity of NGNA has been heavily studied (specially by Amgen – as per Steve Elliot's conversation) since it was originally thought that it would cause development of blocking antibodies since it is not present in humans – but it has not).

HIV 3D IMAGE

This first-ever 3-D image gives scientists an atomic view of the HIV molecule attaching to an immune cell. First the AIDS virus binds to one location on the cell surface and then it changes shape and binds to another site. What is actually seen here is the first main binding site. The red part is the HIV virus and the yellow section is one part of an immune cell.



HIV blocker

In what could lead to a new AIDS drug, scientists have determined how to block the HIV virus from entering cells.

The protein gp120 that sticks out from the AIDS virus binds like a key penetrating a lock and infects the cells

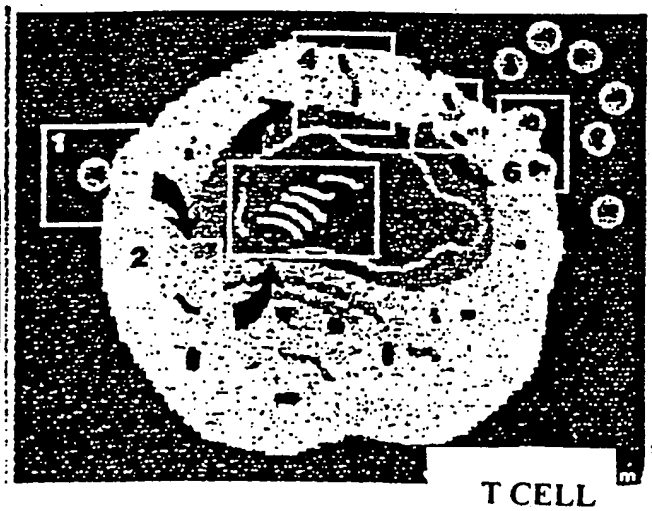
cell

A drug designed to plug any hole in the "key" will prevent infection

cell

HIV BLOCKING

An is schematic



T CELL

CELL ATTACHMENT

CELL ENTERING

A

Reverse Transcriptase

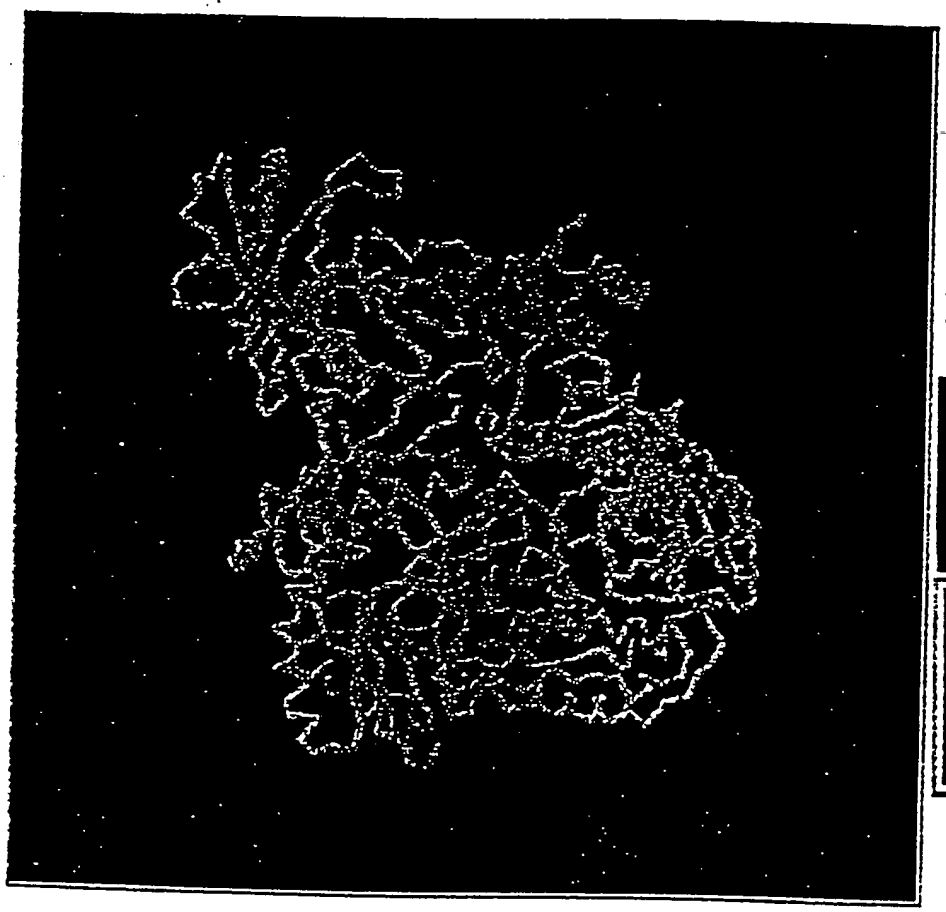
Reverse Transcriptase is the enzyme responsible for replicating the HIV genome. This enzyme contains three catalytic functions:

- 1)RNA directed DNA polymerization
- 2)RNase H activity
- 3)DNA directed DNA polymerization.

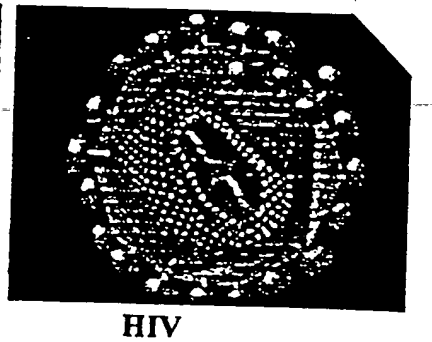
This enzyme is one of the major targets of antiviral therapies for attenuating AIDS.

RT has two subunits, p51(green) and p66(blue)

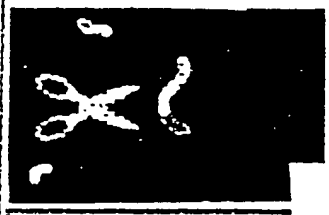
You can click on either subunit of the enzyme to find out more about that subunit.



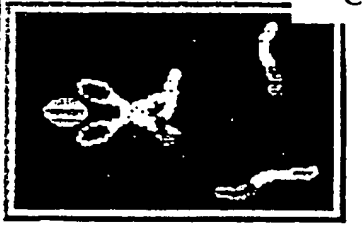
RT REPLICATION



HIV



CLEAVING



PROTEASE

catalytic site

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

June 6, 1997

Ms. Barbara Acello
Director, Patient Welfare
Mankind Institute of Cancer and AIDS Research
8635 Richmond Hwy
Alexandria, VA 22309

Dear Ms. Acello,

Thank you for your recent letter requesting support for research on your product, LUKOR. I want to thank you for the work, thought, and time you have put into your proposal. Experienced and creative scientists form the backbone of the NIH enterprise, helping to advance knowledge that ultimately improves health and medical care.

The hypothesis upon which you base your proposal is highly germane to the development of approaches to treat HIV infection. I believe your idea may be worth pursuing because it is novel and may hold the potential for an approach to development of an HIV therapy, which is so critical to the public health.

I therefore encourage you to look for funding that would enable you to further explore your hypotheses. There are two routes you can follow.

First, you can apply for NIH funding through the R01 process by filling out the PHS 398 Grant Application (the form is on the NIH Home Page at http://www.nih.gov/grants/funding/phs398/phs398.html), keeping in mind that preliminary data would be needed to convince your peers in the review process of the soundness of your hypothesis. I want you to also be award that all proposals for projects with a cost of greater than $500,000 must receive approval by an appropriate institute prior to submission.

The other possibility you can explore is applying for a grant responding to specific initiatives of our Division of AIDS. You can learn about those initiatives by looking at the NIAID Home Page (which can be accessed through the NIH Home Page from the World Wide Web). In addition, to discuss your specific proposal to see if the Division of AIDS would accept it as an unsolicited application, you should talk to Dr. Carl Dieffenbach, Associate Director of Basic Sciences Program, Division of AIDS at 301 496-0637.

If you need additional information about applying for NIH grant support, you can also call 301 435-0714.

Again, thank you for bringing this concept to our attention. I wish you success in obtaining support to pursue it.

Sincerely,

Lawrence Deyton, MSPH, MD
Acting Director
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases

cc: Dr. McGowan, Deputy Director, NIAID
Dr. Carl Dieffenbach, DAIDS

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of Allergy
and Infectious Diseases
Bethesda, Maryland 20892
2C30A Solar Bldg.
(301) 496-0636

August 20, 1997

COPY

Yash Sharma, M.D.H.
Medicine and Applied Sciences, Inc.
8233 Old Court House Road
Suite 102
Vienna, VA 22182

Dear Dr. Sharma:

I've had an opportunity now to review the written material on LUKOR you provided during your visit with me on 8/5/97 and am providing the following comments at your request:

Biological data: The *in vitro* efficacy and lack of cytotoxicity in PBMCs is impressive. Assays seem to have been adequately performed, but results are tempered somewhat by a lack of a defined dose-response relationship for your product. The efficacy in CEM-TART cells is less pronounced and not terribly consistent between the two assays shown. The effects on virus binding, protease, and RT are less marked than on intact cells and may suggest these targets play only a minor role in the mechanism of drug action. It wasn't clear from the material I reviewed how the pre- and post-HPLC purified materials compared in activity.

Analytical data: I'm not sure how much useful information you've gotten from these efforts. I'm unconvinced that a crude lysate would elicit only a few peaks on HPLC, particularly since multiple peaks were seen during the LC portion of the LC-MS study. I suspect re-examination using different solvent conditions and columns would provide a different picture. I don't feel the HPLC profiles can be counted on to provide much information regarding compound size; the elution profile from a reverse-phase column typically tells more about a compound's relative hydrophobicity (although size does indeed factor in). The UV spectra of column fractions don't help much in elucidating structure, nor can they be expected to give more than general suggestions as to potential chromophores present. The mass spectra do suggest low molecular weight species, although the inconsistency in spectra between samples is troublesome. I don't understand how mass spec results could lead one to suggest the absence of nitrogen or the presence of lipid-like structure in a molecule. 5,6-Dimethyldecane may give a mass spectra similar to that of your product, but this compound would not be expected to show a strong UV absorption (no double bonds or aromaticity) and thus seems unlikely to be the source of the large peak seen by HPLC. NMR might indeed give you useful structural info, but I would hesitate to proceed if I had doubts about the substance's purity. Conducting a peak homogeneity test during an HPLC run was suggested to me by others as potentially useful – you monitor at multiple wavelengths and see if the absorbance ratios change across the peak of interest. They shouldn't if the peak represent a single entity.

I hope these comments prove useful to you. I'd rather not try to second guess what additional studies might be required of you when you meet with FDA. The data suggests the presence of a powerful inhibitor of virus replication, but it's unclear where that activity resides. I'm interested in hearing how your efforts are received by FDA, but cannot offer any type of endorsement of your product (or that of any other sponsor). If you have any questions regarding these comments please don't hesitate to call.

Sincerely,

Steven R. Turk

Steven R. Turk, Ph.D.
Therapeutic Research Program
Division of AIDS

cc: C. Dieffenbach

Pre-IND Meeting Agenda
September 18, 1997

Company: Medicine and Applied Sciences, Inc., Alexandria, VA
Product: Lukor (extract from baboon leukocytes)
Clinical indication: AIDS

I. Introduction - David Finbloom, M.D., Director, DCB

II. Presentation - Yash Sharma, MDH, MAASI, Inc.

III. Comments by CBER representatives
- A. Animal use issues - Carolyn Wilson, Ph.D., DCGT
- B. Product manufacturing issues - Kathleen Clouse, Ph.D., DCB
- C. Product characterization issues - Malcolm Moos, M.D., Ph.D., DCGT
- D. Pre-clinical testing
  1. In vitro testing - Kathleen Clouse
  2. In vivo (Pharm/Tox) issues - David Essayan, M.D., DCTDA
- E. Clinical trial issues - Ilana Fogelman, M.D., DCTDA
- F. Advertizing/promotional issues - William Purvis, OELPS

IV. Concluding remarks/recommendations - Dr. Finbloom

7-10-7[illegible]

Lukor

David Flabloom	DCB/FDA/CBER
KATHLEEN CLOUSE	DCB/FDA/CBER
Malcolm Moos	DCGT/FDA/CBER
David Essayan	DCTDA/CBER
Carolyn Wilson	DCGT/CBER
William V. Purvis	APLS/OELPS/CBER
Catherine A. Miller	APLS/OELPS/CBER
[illegible] Muhammad	Abundant Life Clinic
ILANA FOGELMAN	CBER/OTRR/DCTDA
EARL Dye	CBER/OTRR/DARP
Parkash Gill MD	USC School of Medicine. Los Angel
Yan Sh[illegible]	MAAS[illegible] V[illegible]

Chimp research may help AIDS vaccine development

February 2, 1999
Web posted at: 8:50 p.m. EST (0150 GMT)

From Medical Correspondent Elizabeth Cohen

CHICAGO (CNN) -- The discovery announced this week that humans contracted the HIV virus from chimpanzees may help in the development of a vaccine to protect humans from getting infected, say researchers at the 6th Conference on Retroviruses and Opportunistic Infections.

Unlike humans, when chimps are infected with the primate version of HIV, they dc AIDS.

"If we could identify what that factor is -- and there's no guarantee we will, but if w one could direct your vaccine to elicit a response of that particular factor that is prot said Dr. Anthony Fauci of the National Institutes for Health.

The vaccine effort could use the help. After years of research, only one vaccine is ir clinical trials, and many experts think it will not succeed.

CNN's Elizabeth Cohen reports on the effort to find an HIV vaccine for humans

Real	28K	80K
Windows Media	28K	80K

While scientists have been able to come u effective drugs, such as AZT, to treat pec they have had a difficult time finding a va prevent HIV infection.

There are several reasons for the delay in vaccine: The first is money -- vaccines ar most urgently in the poorest countries.

"Massive numbers of vaccines would be needed and companies are concerned, 'wl pay for that,'" said Dr. Seth Berkley of the International AIDS Vaccine Initiative.

The slow progress on a vaccine can also be attributed to science.

"(HIV) is a retrovirus, and one that has the capability of

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inserting itself into a gene – into the genes of our own cell and hiding there essentially indefinitely, so it shields itself from the surveillance of the body's immune system," Fauci said.

There are also safety problems associated with testing a vaccine for a deadly disease.

A research team in Chicago wants to use a live attenuated vaccine. But when used kills them.

"But essentially, the answer is only going to be how a vaccine acts in a human beir acts in an animal," said Gordan Nary of the International Association of Physicians Care.

Nary says one option is to try the vaccine on terminally ill cancer patients, who hav He said the Food and Drug Administration has agreed to hear the proposal.

- Study: One-week trea mother-to-child HIV trans
- AIDS activist Mary Fish anti-HIV treatments
- Drug combinations ch face of pediatric AIDS
- AIDS virus came from doctors conclude

Related stories:

- Study: HIV virus becomes unreachable early in infection - July 20, 1998
- Human HIV vaccine uncertain after monkey vaccine fails - July 2, 1998
- AIDS vaccine trials: a moral and ethical challenge - June 29, 1998

Related sites:

Note: Pages will open in a new browser window

- 6th Conference on Retroviruses and Opportunistic Infections
- National Institutes of Health
- International AIDS Vaccine Initiative
- International Association of Physicians in AIDS Care

External sites are not endorsed by CNN Interactive.

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Today on CNI

Subject: URGENT
Date: Sun, 07 Feb 1999 13:10:48 -0500
From: minarcik <minarcik@gate.net>
To: Anthony Fauci <af10r@nih.gov>, "Yash P. Sharma" <ysharma@worldnet.att.net>

Dear Dr. Fauci:

I have taken the liberty of pasting one of your quotes from a recent CNN news release regarding chimp vaccines:

"If we could identify what that factor is-- and there's no guarantee we will, but if we can-then one could direct your vaccine to elicit a response of that particular factor that is protecting them."

We may have found what "that factor" is.

"that factor" is carbohydrate found in mammals which do not get AIDS, notably the baboon, but also chimps, cats, dogs, rats, and horses. Humans however have only trace amounts of this carbohydrate.

About two years ago MAASI labs prepared an extract of baboon lymphocytes, which had remarkable anti HIV properties in vitro (see enclosed Abst-105.doc). The active "peak" in this cellular extract, which we called ANKA (Aids Natural Killing Agent), we have recently discovered to be a very specific cell surface carbohydrate using NMR and other methods.

This carbohydrate is not found in species which are susceptable to AIDS. Animals which produce thi compound however, are reported to be immune from AIDS. This concept concurs with your observation as noted in the CNN release. Please read our enclosed other documents too. Lukor update.doc, and Board.doc. , and help us to develop this safe, natural, anti-retroviral compound/vaccine.

John R. Minarcik, MD

HÔPITAL GÉNÉRAL JUIF - SIR MORTIMER B. [illegible]
THE SIR MORTIMER B. DAVIS - JEWISH GENERAL HOSPITAL

INSTITUT LADY DAVIS DE RECHERCHES MÉDICALES
LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH

July 31, 1998

By fax: (703) 360-6248

Dr. Yash P. Sharma, President
Medicine and Applied Sciences Inc.
8653 Richmond Hwy
Alexandria, Virginia 22309

Dear Dr. Sharma,

Thank you for giving me the opportunity to read and review the dossier on "LUK0R". I spent the equivalent of one working day reviewing this file. My conclusions are as follows:

1. The presence of a unique substance in baboon blood that may protect against retroviral infection represents a reasonable hypothesis.
2. LUK0R is a leading candidate in regard to helping to explain the observed resilience of baboons to retroviral disease.
3. LUK0R may potentially possess a wide array of biological activities, some of which may have relevance in regard to HIV disease and to the ability of HIV to replicate in infected target cells.
4. The activity of LUK0R against HIV-1 replication may potentially be manifested through either direct or indirect mechanisms. Given the fact that different levels of anti-viral activity have been reported in different cell types, and at different drug concentrations, it is conceivable that the mechanism of action may not be identical in all cell types analyzed.
5. The direct inhibitory effects of LUK0R in regard to viral protease and reverse transcriptase activities are modest. Conceivably, the viral integrase enzyme may also be a target for LUK0R. The mechanisms of anti-viral enzyme activity demonstrated to date may occur through indirect mechanisms. Further work in this area should be performed.
6. The effects of LUK0R may be manifest in certain cell systems through signalling mechanisms and other pathways. For example, research is needed in regard to whether or not LUK0R may affect expression levels of the CCR4 and CCxR5 co-receptors, as well as that of CD4 itself in various cell types.
7. The effect of LUK0R may also be due to ability to impact on viral regulatory proteins, e.g. Tat, by interacting with NF- κB and other cellular factors known to be involved in control of viral replication.
8. A possible effect of LUK0R in regard to potentiation of specific and non-specific aspects of anti-viral immunity should be explored. Conceivably, LUK0R may serve to enhance natural killer (NK) cell activity or to amplify numbers of NK cells or of cytotoxic T lymphocytes with activity against HIV-infected targets

Thank you again for giving me the opportunity to review the LUK0R file. I look forward to positive interactions with you and MAASI.

Sincerely,

Mark A. Wainberg, Ph.D.

Subject: Re: Neu5N-Glycolyl
Date: Fri, 30 Apr 1999 13:20:53 -0500
From: gjboons <gjboons@ccrc.uga.edu>
Organization: ccrc
To: Yash Sharma <ysharma@worldnet.att.net>

Dear Yash

This is the content of the attached file.

Geert-J*an

Many proteins and lipids are co-valently bound to oligosaccharides and these conjugates are named glycoproteins and glycolipids. Sialic acids are the most abundant terminal components of oligosaccharides of mammalian glycoproteins and glycolipids and the most commonly observed linkages are Neu5Aca(2Æ3)Gal, Neu5Aca(2Æ6)Gal, Neu5Aca(2Æ8)Neu5Ac, and Neu5Aca(2Æ9)Neu5Ac. The sialic acid moieties of glycoproteins are involved in many critical recognition processes.
The enzymes that are involved in the biosynthesis and incorporation of sialic acid tolerate modified substrates in particular on nitrogen.[1] Thus, it is to be expected that N-glycolyl neuraminic acid will be incorporated into the oligosaccharide chain of glycoprotein and glycolipids. On the other hand, sialidases, which are enzymes that cleave glycosides of sialic acid, have reduced activities with modified N-acyl groups.[2] Thus, it is to be expected that N-glycolyl neuraminic acid, which is incorporated in glycoproteins and lipids, will have a considerable half-life time.
Some of the anti-AIDS properties of N-glycolyl neuramic acid may be based on modifification of glycoproteins.[3] For example, studies of Sharma and co-workeres indicate that N-glycolyl neuramic acid provides long-term protection to CD+4 cells. It is well known that sialic acid plays important roles in immunological reactions. Several important functions of immune cells can be affected by subtle changes in the extent of sialylation and for example, there is evidence the stimulation of CD4+ cells by APC are influences by cell-surface sialylation. Modification of the structure of sialic acid may impact these recognition processes.
It has also been established that N-glycolyl neuraminic acid is a protease inhibitor. Many protease inhibitors require the amino acid serine. It can be speculated that the glycolyl moiety of N-glycolyl neuraminic acid mimics the side-chain of serine and therefore acts as an inhibitor.
Neuraminic acid of glycoproteins are also important in particular viral infections in particular the influenza virus.[4] The influenza virus has two major surface proteins, heamagglutinin and neuraminidase. Heamagglutinin mediates the binding of the viron to sialic acid-containing receptors on the surface of the target cells in the respiratory tract. It also mediates the fusion of viral and cell membrane. The neuraminidase protein facilitates the release of newly formed virons from the host cell surface and prevents aggregation by cleavage host terminal sialic acid. Glaxo-Wellcome has developed a Neu5Ac analog, which inhibits the neuraminidase. This compound shows great promise for the treatment of flu.

1. a) R.E. Kosa et al., Biochem. Biophys. Res. Commun., 190, 914, (1993); b) S.L Shames, Glycobiology, 1, 187 (1991); c) C-H. Lin, J. Am. Chem. Soc., 114, 10138 (1992); d) H. Kayser, J. Biol. Chem., 267, 16934 (1992)
2. R. Drzeniek, Hisochem. J., 5, 271 (1973)
3. Y. Pilatte, Glycobiology, 3, 201 (1993)

Subject: RE: [Fwd: [Fwd: NeuGc]]
Date: Thu, 11 Mar 1999 14:11:35 -0500
From: Steven Turk <STURK@niaid.nih.gov>
To: 'Yash Sharma' <ysharma@worldnet.att.net>
CC: Steven Turk <STURK@niaid.nih.gov>

Will do. Thanks.

-----Original Message-----
From: Yash Sharma [mailto:ysharma@worldnet.att.net]
Sent: Wednesday, March 10, 1999 8:48 PM
To: Steven Turk
Subject: [Fwd: [Fwd: NeuGc]]

Dear Dr. Turk:

Here is information on Lukor active molecule. you can buy it directly from Sigma for you testing and share the results with MAASI as agreed under our existing CDA.

Yash

Subject: re: HIV immunity factor in chimps-LUKOR sugar
Date: Wed, 17 Feb 1999 17:56:45 -0500
From: Steven Turk <STURK@mercury.niaid.nih.gov>
To: "'ysharma@worldnet.att.net'" <ysharma@worldnet.att.net>
CC: Steven Turk <STURK@mercury.niaid.nih.gov>,
Carl Dieffenbach <CDieffenba@mercury.niaid.nih.gov>

Dear Dr. Sharma:

Thank you for the summary information on LUKOR which you provided to 12 NIH staff members on February 6. The majority of the individuals receiving your message are members of the Division of AIDS and are cognizant of your regular interactions with myself and Dr. Dieffenbach regarding your efforts to develop this product. Informational updates such as that which you provided as a matter of process are forwarded to the appropriate staff here for a follow-up response -- myself in this case. Since I'm already quite familiar with this topic and the material you provided, I have little in terms of new insight to offer at this time. The observation that humans may have contracted the HIV virus from chimpanzees was indeed interesting, but seems to offer little in furthering the development of this particular product. A "chimp factor" may indeed lessen the disease symptoms seen in chimps vs. humans after virus infection, but clearly, if the virus originated with chimps, it was actively replicating in chimps in the presence of this "factor" or could not have continued to be propagated as an infectious agent.

We hope you have been receiving useful advice from us for advancing your drug development plans for this material. Investigators such as yourself naturally carry the primary responsibility for developing and commercializing new therapies. The Division assists investigators when it can via well established grant mechanisms to provide funding and by selective use of our contract resources to address certain drug development issues. These latter resources unfortunately are limited and generally are devoted to development of well characterized, pure materials. You already have taken all the appropriate steps required in seeking our assistance. We have offered to work with you to initiate in vitro evaluation of the newly identified active component of LUKOR, but remain hesitant at this time to offer substantial assistance in the development of the homogenate as a therapeutic agent. Discussions about potential clinical trial assistance from the Division would be premature at this time without substantial documented evidence that FDA is likely to issue an IND for the study of this material. We know you have been making progress in this area and encourage you to continue your discussions with FDA.

Thank you again for the information you provided. We hope the planned in vitro evaluations the Division will be doing for you proceed well and look forward to hearing from you as your IND negotiations with FDA move forward.

Steven R. Turk, Ph.D.
Division of AIDS, NIAID, NIH
Tel (301) 435-3771 Fax (301) 402-3171
e-mail: st15m@nih.gov

-----Original Message-----
From: Yash Sharma [mailto:ysharma@worldnet.att.net]
Sent: Saturday, February 06, 1999 6:35 PM
To: bl17u@nih.gov; cdc; ch25v@nih.gov; cp22n@nih.gov; fb10c@nih.gov; gfolkers@nih.gov; jk31e@nih.gov; jk38m@nih.gov; jm79q@nih.gov; mm52s@nih.gov; ns18p@nih.gov; Picaboo32@aol.com; rh25v@nih.gov;